EFFICACY OF RESIN-BASED MATERIALS

AGAINST EROSIVE-ABRASIVE

WEAR IN VITRO

by

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DEDICATION

To the people who helped to turn this dream into a reality: my parents, my mentors, my colleagues, my family, and my husband.

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INTRODUCTION

Increasing prevalence of tooth erosion has been observed in many countries, in both children and adults.[1] Larsen defined dental erosion as a chemical process that involves the dissolution of enamel and dentin by acids not from bacterial origin, due to the creation of undersaturated conditions with respect to tooth mineral.[2]

The acids responsible for dental erosion can be classified as intrinsic or extrinsic depending on their source.[3,4] The extrinsic acids are mostly from acidic foods and beverages, medication, or acidic fumes in chemical or galvanic factories.[5] The main intrinsic source for acids is the gastric juice[6] mainly composed of hydrochloric acid. Disorders such as anorexia and bulimia nervosa, gastroesophageal reflux disease (GERD), the consequences of chronic alcoholism and binge drinking [7, 8] have been linked with frequent direct contact of teeth with gastric juice whose pH can be as low as 1. Repeated exposure will result in an acidic dissolution of dental hard tissues. Intrinsic dental erosion is often associated with softening of the surface[9] accompanied by severe irreversible tooth damage.[10] There is a synergistic effect between erosion and abrasion in the process of wear of dental hard tissues.[11]

Several studies have demonstrated the potential of tooth brushing to produce additional damage to the softened eroded enamel and dentin.[12-14] *In situ* and *in vitro* studies have shown that the susceptibility of dental surfaces to toothbrushing abrasion increases with increasing abrasivity of the dentifrice.[11,15] Radioactive enamel abrasivity (REA) and radioactive dentin abrasivity (RDA) are laboratorial tests used to measure the relative abrasivity of toothpastes. In the enamel, erosion will cause demineralization of the mineral phase, mainly consisting of impure hydroxyapatite and fluorapatite crystals. The crystals then become ill-organized and more easily affected by acid demineralization. In dentin, demineralization starts on apatite crystals at the interface between intertubular and peritubular dentin, and once the amount of exposed collagen increases, the speed at which the demineralization happens will decrease.[16]

In agreement with the principles of minimally invasive dentistry it has been recommended that, in the management of dental erosion, special attention should be given to early diagnosis and preventive measures to avoid the need for complex and extensive rehabilitation.[17] Different strategies have been proposed to prevent and inhibit the progression of tooth wear. In 1972, Graubart et al. in an *in vitro* study demonstrated that 2% sodium fluoride offered protective effect against erosion.[18] Since then, the effect of different fluoride formulations on dental erosion has been thoroughly investigated.[19] Fluoride agents at high concentrations, different formulations and in different vehicles have been shown to increase abrasion resistance and decrease the progression of enamel and dentin erosion *in vitro*[20] and *in situ*.[21] In the presence of acidic conditions, fluoride will increase the resistance of the tooth surface to erosion rather than fostering remineralisation.[22] The action of fluoride can be explained by two different mechanisms. The first is mainly attributed to a precipitation of a CaF_2-like material that will be dissolved under erosive conditions and temporarily protects the underlying enamel.[21] The second is when polyvalent metal fluorides are used and a more acid resistant metal-rich surface enamel layer is formed.[23] In both situations the protection has limited duration and requires repeated applications of the fluoride agent.[24] The durability of the anti-erosive effect of topically-applied fluorides is limited, ranging from a few seconds (75s)[25] to a few minutes (3.5–18 min),[26, 27] to over one and one-half hours[28] after a single application.

Another strategy proposed is the application of a mechanical barrier to hinder the direct contact of erosion causing acids to enamel and dentin.[29] The use of resin-based dentin bonding agents to protect dentin from erosion has also been reported.[30] *In vitro*, [31] *in situ*, [32] and *in vivo* [33] studies have been carried out to investigate the protective effect of fissure sealants and dentin bonding agents against erosion and abrasion and showed a significant protective effect. An *in vitro*[34] study compared the effect of repeated fluoride mouthwash applications and an adhesive against erosion and abrasion and concluded that the adhesive delayed the surface wear when compared to the fluoride mouth rinse.

Azzopardi et al. [31] investigated the use of a desensitizing agent and a dentin adhesive to protect against an erosive and abrasive wear challenge. The results showed that both materials were effective in protecting dentin against further wear when compared to a control, although the dental adhesive provided more protection than the desensitizing agent. Conversely, an *in situ*[32] investigation examining both products showed that the desensitizing agent provided statistically significantly more protection than the dentin adhesive. A clinical study on adult subjects with palatal surface wear exposing dentin, concluded that a desensitizer agent offered some protection against tooth wear up to three months.[35] With the aim of investigating a material that could prevent teeth wear for longer periods, a fissure sealant was used to coat worn palatal surfaces of anterior teeth in adult patients.[33] This clinical study showed an apparent protection even after the sealant was lost for up to 9 months. The permanence of tags of fissure sealant even after the disappearance of the surface layer can be the explanation for this event.

More recently an *in vitro* study[36] investigated the application of a resin infiltrant on eroded enamel subjected to erosive challenges. In this study enamel samples were previously softened in HCl and then treated with four different resin-based materials including an infiltrant. The specimens were then exposed to erosive cycling mimicking intrinsic erosion and analyzed by a profilometer. The results showed that the resin infiltrant was able to penetrate the enamel with or without acid etching and was able to protect against the progression of the erosive attack. Even though the results were promising, this study did not investigate the association between erosive and abrasive challenges on the performance of the infiltrant or the effect of this material on dentin erosion.

Until now information concerning the efficacy of a resin infiltrant to protect enamel and dentin against further erosion and abrasion is still scarce. In the present project, it was hypothesized that protection against further erosion and tooth brushing abrasion results from an interaction of factors including the characteristics of the material used, the level of abrasivity of the dentifrice and time. Hence, our main objective was to use an *in vitro* model to better understand the protective effect of different materials against dental erosion and tooth brushing abrasion performed with simulated dentifrices of different abrasive levels measured over two time periods.

OBJECTIVES

The objectives of this *in vitro* study were: 1) To evaluate the protective effect provided by three resin-based materials (pit & fissure sealant - Helioseal® Clear Ivoclair, USA; dentin sealant - Seal & Protect™,Dentsply, USA, and resin infiltrant - Icon®,DMG, Germany), one fluoride varnish (Duraphat® Varnish, Colgate, NY, USA) and no treatment (control group) against dental erosion and tooth brushing abrasion on enamel and dentin; 2) To evaluate the influence of the abrasive level of the dentifrice on the protective effect of different materials; 3) To evaluate the influence of time on the protection yield by different materials on enamel and dentin.

HYPOTHESES

Null Hypotheses

1. There is no difference in the efficacy of the treatments to protect against erosion and tooth brushing abrasion for enamel and dentin.
2. Different abrasive levels will not affect the efficacy of the treatment materials to protect against erosion and abrasion in enamel and dentin.
3. Time will not influence the protective effect of the treatment materials for enamel and dentin.

Alternative Hypotheses

1. There will be at least a difference in efficacy among treatment materials in protecting enamel and dentin from erosion and abrasion.

2. The abrasive level will affect the ability of treatment materials to protect enamel and dentin from erosion and abrasion.

3. Time will influence the ability of the treatment materials to protect enamel and dentin from erosion and abrasion.

REVIEW OF LITERATURE

DEFINITION AND ETIOLOGY OF DENTAL EROSION AND ABRASION

Erosive tooth wear is a non-carious loss of tooth structure that can have various etiologies encompassing attrition, abrasion, abfraction and erosion. More often than not these processes occur concomitantly with a synergistic effect. There is a general agreement that acids responsible for dental erosion also potentiate the deleterious effects of attrition, abrasion, and abfraction.[9, 37, 38] For the purpose of this study more emphasis will be given to erosion and abrasion and the association of both.

Dental erosion has been defined as the result of a progressive dissolution and loss of tooth structure due to an acidic exposure without bacterial involvement when the surrounding conditions are undersaturated with respect to tooth mineral.[2] Acid introduced into the oral cavity will dissociate in saliva into hydrogen ions and anions, decreasing the pH of the oral environment and thus making it undersaturated in relation to the dental surfaces. Hydrogen ions will then attack the tooth structure and combine with the carbonate and/or phosphate molecules, releasing calcium ions from the apatite crystals.[39] Dental erosion is a multi-factorial condition and according to the source of the causative factors it may be divided into extrinsic and intrinsic types.[40, 41]

Acids of extrinsic origin are mainly from the diet. Carbonated beverages, fruit juices, smoothies and some alcoholic drinks have erosive potential.[42, 43] Furthermore, some of these acids such as citric acid, are also chelating which potentiates their erosive nature.[44] Other extrinsic sources of acids that can affect the dentition include the work environment (chemical industry and wine tasters), exposure during sports activities (e.g. swimming pool water),[45] and medications.[46] Gastric juice entering the oral cavity is the only intrinsic source for acids.[6] Disorders associated with persistent vomiting and regurgitation or gastroesophageal reflux have been linked to frequent direct contact of teeth with hydrochloric acid whose pH can be as low as 1.[8, 46] Many conditions are associated with the movement of the gastric acid from the stomach to the mouth. Vomiting is described as a forceful expulsion of the stomach contents through the mouth resulting from many organic and psychosomatic disorders such as pregnancy, anorexia and bulimia nervosa.[47] Regurgitation is the involuntary movement of gastric juice from the stomach to the mouth observed with gastroesophageal sphincter incompetence, with increased gastric pressure and volume.[8] Gastroesophageal reflux disease (GERD) is the persistent backflow of the stomach contents past the lower esophageal sphincter.[10] Gastric fluid of healthy patients and patients with gastroesophageal reflux disease have been shown to have a wide variation in pH ranging between 1.2 and 6.7 [48] and titratability (mmol OH-/L) ranging between 83 and 27.[49] The lower pH and titratable acidity of gastric acid significantly increases its erosive potential when compared to carbonated drinks.[48] Intrinsic dental erosion is often associated with softening of the dental surface[9] accompanied by severe irreversible tooth damage.[10] The palatal surfaces of the upper anterior teeth seem to be more affected by the acid once it reaches the mouth.[50]

Dental abrasion is defined as the wear produced by interaction between teeth and other materials. Although essential to maintaining good oral health, tooth cleaning is considered the most common cause of abrasion.[38] With respect to the development of tooth abrasion, frequency, duration and force of brushing and the relative dentin abrasivity of the toothpaste are the most important factors;[51] however, characteristics of the toothbrush can modulate the abrasive potential of the toothpaste.[52] Data from clinical and *in vitro* studies have shown that in Western populations the major abrasive agent is toothpaste.[51] Although the presence of the abrasive in the toothpaste is necessary to remove stain from the teeth,[53] a balance between efficacy and harm is achieved by limits on the abrasivity of a toothpaste set by the International Organization for Standardization (ISO). The tests use a radiotracer methodology which provides radioactive dentine abrasivity (RDA) and radioactive enamel abrasivity (REA) values compared to a reference abrasive giving scores of 10 or 100, respectively.[11, 13, 51] Since sound dentin is more easily affected by abrasion than enamel, the RDA value is used as the main parameter to characterize the abrasivity of toothpastes.[38] It has been shown that the amount of tooth wear is insignificant when toothbrushing is performed using normal force, amount of, and standard toothpaste.[51]

INTERPLAY BETWEEN EROSION AND ABRASION

Erosion is responsible not only for direct loss of hard tissue but also to render dental surfaces more vulnerable to mechanical wear.[54] *In vitro*[55] and *in situ*[56] studies have shown that eroded enamel and dentin are more susceptible to toothbrush abrasion. A decrease in microhardness has been demonstrated for eroded enamel and dentin. Furthermore, there is an inverse correlation between Vickers microhardness values and the susceptibility of enamel to toothbrushing abrasion. [14] When enamel is exposed to acid, part of the mineral is etched away leaving a soft mineral surface. [57] Results from *in vitro* studies have shown that softened enamel is more easily affected not only by toothbrushing with paste, [58, 59] but also by toothbrushing without paste, [60] and by friction with the oral soft tissues.[61, 62] A loss of 0.25–0.5 µm of tooth surface has been observed during toothbrushing after an erosive challenge which is comparable to that experienced during drinking an acidic beverage. [59,63]

When dentin is exposed to acid, the dissolution is first observed at the peritubular and intertubular junction followed by loss of the peritubular dentin.[64] The continuation of this process will result in the formation of a layer of demineralized collagenous matrix which prevents further acid diffusion and mineral release.[65, 66] This layer is composed mainly of cross-linked, fibrous collagen and appears to be rather resistant to brushing; [67] however, several studies showed an increased loss of eroded dentin after brushing treatment.[68-70] Similar to sound dentin, abrasion of eroded dentin increases as the RDA value of the abrasive in the dentifrice increases and can be modulated by characteristics of the toothbrush.[71]

EPIDEMIOLOGY OF TOOTH EROSION

Research into the prevalence and etiology of dental erosion has been extensive in the last few decades; however, reported prevalence varies widely due to use of different scales and scoring systems and compositions of the cohort groups.[72] Prevalence data have shown that erosive tooth wear is a common condition and is growing steadily. Dental erosion is currently estimated to occur in 2-56 percent of the population, varying depending on the age and location of the sampled population.[73, 74] Large variation in the prevalence of dental erosion was found among different age groups. For younger children aged 2-9 years, the prevalence ranged between 6 and 50 percent. The age group that showed the highest prevalence, 11–100 percent, was older children with ages from 9 to 17 years. For the older age group adults from 18 to 88 years, the prevalence was between 4 and 83 percent.[75] A review published by Johansson et al.[76] reported the prevalence of dental erosion in adults varied from 11 to 77 percent, 1 to 53 percent in adolescents and 1 to 34 percent in children. Furthermore, they found that dental erosion, particularly palatal damage of the upper front teeth, is common among children and young people from different countries. Increasing levels of tooth wear have also been significantly associated with age.[77]

A study investigated the prevalence, distribution and severity of dental erosion and its association with lifestyle, oral and general health in young adults in Sweden. The results showed that 25 percent of the subjects had no erosion, 75 percent had erosion and 18 percent had extensive erosion. The occlusal surface of the molars was more affected with 74 percent of the lesions followed by 7.3 percent on the palatal and 3.8 percent on the buccal surfaces of maxillary incisors, respectively. A relationship between erosion, behavioral factors, oral health and body mass index[72] was also reported.

Few studies have been published concerning the prevalence on dental erosion in the United States. To measure the prevalence of erosion of the upper permanent incisors of 11-13 year old children in the United States and the United Kingdom, 129 and 125 subjects were examined, respectively. The prevalence of erosion in the United States was 41 percent and 37 percent in the United Kingdom; however, the difference was not statically significant. The majority of the subjects presented lesions confined to enamel.[78] McGuire et al.[79] investigated the prevalence of erosive tooth wear in children aged 13-19 years in the United States and found that 45.9 percent showed erosive wear in at least one tooth. Higher prevalence was found for maxillary teeth and for males. Okunseri et al. in 2011,[80] examined the relationship between the consumption of juices, drinks, milk and erosive wear in children in the United States. The results showed that the prevalence of erosive wear was highest in children aged 18–19 years (56 %), males (49 %), and lowest in blacks (31 %). Regular consumption of apple juice was associated with erosive wear. A convenience sample of 307 children aged 12-17 years from San Antonio, Texas was examined in a study focusing on the prevalence of erosive wear in children. The results showed a prevalence of 5.5 percent, the lesions were rather confined to enamel, and associated with consumption of soda drinks.[81]

The average annual incidence data of erosive wear in schoolchildren and adolescents values range between 3.5 and 18 percent, depending on the initial age of the examined sample. Incidence data are scarce in adults with values ranging from 5 percent for the younger and 18 percent for older age groups. Overall, males present more erosive tooth wear than females.[82] A longitudinal study from Germany showed that the number of lesions nearly doubled during the studied period; erosion into dentin on at least one primary tooth increased from 18 to 32 percent and on the first mandibular molars from 4 to 9 percent.[83] The same trend could be observed in the UK where 27 percent of the 12-year-olds had developed new or more advanced erosive damage at age 14; from the age of 12 to 14 the percentage of lesions into dentin increased from 5 percent to 13 percent. For lesions confined to enamel the numbers were 56 to 64 percent.[84, 85]

TREATMENT

Fluoride

The effect of different formulations of fluoride to prevent dental erosion has been thoroughly investigated, [19,86] however without controversy. Whilst there is an understanding that fluoride is able to interfere and modify the erosive process[87] an *in vitro* study has shown that fluoride did not have preventive effect against erosion.[88] The action of fluoride can be explained by two different mechanisms. The first is mainly attributed to a precipitation of a CaF_2-like material that will be dissolved under erosive conditions and temporarily protect the underlying enamel.[21] The second is when polyvalent metal fluorides are used and a more acid resistant metal-rich surface enamel layer is formed.[23] In both situations the protection has limited duration and requires repeated applications of the fluoride agent.[24]

The main purpose of the fluoride application is to halt the progression of erosion via reduction of the solubility of the tooth surface.[89] For treatment of erosive lesions, applications of fluoride in high concentrations seem to be more appropriate.[54] High concentration fluoride is believed to promote formation of calcium fluoride (CaF_2) on the enamel surface, which may result in a mineral surface less prone to erosive dissolution.[90]

Fluoride varnishes have been proposed as a preventive management of erosion, not only because they furnish high amounts of fluoride, but also due to their mechanical protective component that could be advantageous in reducing wear progression.[91] Fluoride varnishes are strategically formulated to adhere to the tooth surface, harden in the presence of saliva and slowly release fluoride over time. Sorvari et al. in 1994, first investigated the use of NaF varnish applied for 24 h and removed before an erosive challenge and showed an increase in enamel hardness values and subsequent inhibition of softening.[92] An *in vitro* study examined the effects of NaF varnish applied for 24 h after which it was removed and APF gel applied for four minutes on the erosive wear of enamel of primary and permanent teeth. The samples were exposed to six daily demineralization–remineralization cycles of 5 min of immersion in a cola drink (pH 2.3) and 30 min in artificial saliva during seven days. The results showed that fluoride had no effect on enamel of primary teeth. Although no difference between varnish and gel was observed, they both inhibited erosive enamel loss in permanent teeth.[93] The effect of a single application of NaF/CaF_2 varnish was investigated in an *in vitro* erosion/abrasion cyclic model simulating intrinsic erosion. Human enamel samples were treated with two different fluoride solutions and a fluoride varnish after which they subjected to either erosive cycles using 0.01 M HCl, pH 2.2 for 2 min or erosion–abrasion (120 strokes) cycles. The results showed a limited protection against erosion, but when abrasion was added, no protection was detected.[26] Magalhaes et al.[94] studied the effect of NaF varnish to protect against dentin erosion using *in vitro* erosion/abrasion cycling. Bovine dentin received different fluoride treatments and was then exposed to erosive cycling using a soft drink (pH 2.6) 4 × 90 s per day and to toothbrushing-abrasion 2 × 10 s per day for five days. A significant reduction in dentin tissue loss was seen for the fluoride varnish when compared to placebo varnish, control and fluoride solutions.

In all the studies described above the fluoride varnish was removed before the specimens were exposed to an erosion or erosion/abrasion challenge; therefore the effect of the fluoride relied only on the strengthening mechanisms provided by the chemical interaction with the dental tissues. Additionally, it has been proposed that besides the chemical effect (fluoride uptake and formation of calcium fluoride deposits) fluoride varnish can also have a coating effect that forms as a mechanical barrier against acidic challenges. An *in vitro* study[91] demonstrated that fluoride varnish left on the tooth surface was able to prevent surface loss and withstand erosive challenge up to 70 min. The same effect was investigated *in situ*[28] using erosion combined with toothbrush abrasion cycling. The results showed only partial protection. Even though groups treated with fluoride varnish showed less surface loss than control groups the protection was time dependent and greatly reduced by the abrasive challenge.

Resin based materials

The use of resin based dentin-bonding agents to protect dentin from erosion has also been reported.[30] *In vitro,* [31] *in vivo*[33] and *in situ*[32] studies have been carried out to investigate the protective effects of fissure sealants and dentin bonding agents against erosion and abrasion and showed a significant protective effect. Sundaram et al.[34] in an *in vitro* study compared the effect of repeated fluoride mouthwash (0.05%) applications and a dentin sealing adhesive (Seal and Protect – Dentsply) against erosion and abrasion.

Human enamel specimens were used and after treatment were immersed in 0.3% citric acid (pH3.2) for 5 min and then exposed to an erosion/ abrasion cycling until 5000 strokes were reached. The mean wear measurements for the dentin sealing was 0.015mm (SD 0.090) compared to 0.127mm (SD 0.150) for fluoride with the groups being statistically different ($p < 0.001$). The results confirmed that Seal and Protect may delay the wear of dentin.

Azzopardi et al.[31] investigated the use of Seal and Protect (desensitizing agent - Dentsply) and Optibond Solo (dentin adhesive - Kerr) to protect against an erosive and abrasive wear challenge. Human enamel was used and subjected to 3000 erosion/abrasion cycles. The amount of wear on Seal and Protect had a mean 24.8 µm and for Optibond Solo it was 1.4 µm with the difference being statistically significant ($p=0.02$). The wear measured on the unprotected teeth was 243 µm and was significantly different from the protected surfaces ($p=0.001$). The results showed that both materials were effective in protecting dentin against further wear when compared to a control with Optibond Solo providing more protection than Seal and Protect. Conversely, an *in situ* investigation[32] examining both products showed that Seal and Protect provided statistically significantly more protection than Optibond Solo. A clinical study of 19 adult subjects with palatal surface wear exposing dentin compared the wear protection of Seal and Protect to uncoated surfaces. Silicone impressions were made at baseline, 3, 6, 9, 12 and 24 month recalls and scanned using a non-contacting laser profilometer. A statistically significant difference in wear between Seal and Protect and control was only observed at three months.[35] With the aim of finding a material that could prevent tooth wear for longer periods, a fissure sealant was used to coat worn palatal surfaces of anterior teeth in adult patients.[33] This clinical study showed an apparent protection even after the sealant was lost for up to nine months. The permanence of tags of fissure sealant even after the disappearance of the surface layer can be the explanation for this event.

More recently an *in vitro* study[36] investigated the application of a resin infiltrant on eroded enamel subjected to erosive challenges. In this study bovine enamel samples were immersed in 0.01 M hydrochloric acid (HCl), (pH 2.3), for 30 s to create a softened eroded surface, and then treated with a pit & fissure sealant (Helioseal Clear), two dental adhesives (AdheSe -self-etching; and Tetric N-bond- conventional adhesive system), and an infiltrant (Icon). They were then exposed to an erosive cycling using 0.01 M HCl (pH 2.3) for 2 min followed by immersion in artificial saliva for two hours, four times a day for five days. Material thickness and surface loss were measured by profilometer. The results showed that all the resin-based materials provided enamel protection against erosive cycling, except for the conventional adhesive.

IN VITRO EROSION/ABRASION MODELS

The increase in the prevalence of tooth wear has also triggered an increase in research to try to understand this process. *In vivo*, *in situ* and *in vitro* models have been used to investigate the process and treatment alternatives for tooth wear. Nonetheless, the number of *in vivo* studies in tooth wear research is limited due to financial constraints and ethical reasons. *In vitro* and *in situ* studies are used more frequently. *In situ* studies have the benefit of incorporating factors that modulate the wear process in the oral environment such as the presence of saliva; however, results can be limited by patient's compliance. *In vitro* models are advantageous since they allow a large sample size to be tested and a considerable number of variables to be examined. Data from *in vitro* studies can be used as preliminary information to guide the researcher on the design of a clinical study. One of the limitations is the impossibility of reproducing all the biological variations in the oral environment that influence tooth wear.[95] Nevertheless, *in vitro* models are designed so that clinical conditions are exaggerated and a worst-case scenario can be tested.[96] Many different models are available in tooth-wear research. They are able to model early stages of the erosion process alone or be incorporated in a combination of erosion and mechanical processes to mimic a more realistic scenario with clinical relevance.

Erosion/abrasion Cycling

A significant interplay exists between chemical and mechanical wear in the oral environment. It is well known that eroded surfaces are more susceptible not only to tooth brushing but also to attrition and even to the friction of the soft tissues. Toothbrushing is a suitable substitute for intra-oral forces even though it may not be applicable to the site and location of erosive wear.[97] Several studies have used the erosion/abrasion models and the number of cycles vary from three[50, 62,98] to 720[99] cycling treatments. Some models use the same number of erosive and abrasive challenges; however, models that use fewer abrasive[96] than erosive challenge are more representative of a clinical scenario since professional bodies typically recommend brushing twice a day.[100] Several factors such as force, number of strokes, type of brush, length of time, the abrasive or other lubricant should be controlled. Studies have found the brushing time for the entire dentition to be between 30-90 s which corresponds to 300-400 brushing strokes[100-102] and 10–15 brushing strokes per tooth.[67]

The duration of the erosive challenge will vary accordingly with the objective of the study and the pH of the erosive agent and ranges from 15 s to 40 min per cycle. Immersion time between 1 and 5 min per cycle is most often used.[96]

Dental Substrates

Enamel and dentin specimens are prepared either from extracted human teeth or bovine teeth, albeit hydroxyapatite disks have also been used. Human enamel and dentin substrates are preferable for in vitro studies; however, they are not always readily available. Bovine teeth can be obtained more easily and can be used as a substitute for human teeth. Human and bovine dentin appears to wear similarly under erosion-abrasion challenges.[11] Though similar to human teeth, enamel from bovine teeth seems to be more susceptible to wear under identical conditions.[103]

For *in vivo* and *in situ* study designs it is important to define the baseline of the specimen during the study preparation. Depending on the measurement technique, the surface of the specimen must be polished and flattened through a standardized polishing protocol. Approximately 100 μm is lost during this procedure[105] and this surface appears to be more affected by acid dissolution.[39]

Type of Acid

Different types of acid are used in erosion-abrasion models and they will be different if the study is modeling extrinsic or intrinsic erosion. For extrinsic erosion, citric, malic, acetic and lactic acids have been used. Dietary acids such as from soft drinks (Coca Cola or Sprite: pH 2.3–3.2), juices (orange, grapefruit, lemon or blackcurrant: pH 3–4), wines (pH 2.9–4.2), acidic candies (pH 2.3–3.1) or sprays (pH 1.9–2.3) are also an alternative.[97] To mimic intrinsic erosion hydrochloric acid has been used in different concentrations. The pH of the gastric juice ranges from 0.9 to 1.5, but in the mouth, it is never below 1.5 due the properties of the saliva present.[106] Several other variables may affect the extent of the erosion and should be controlled such as temperature, agitation and concentration of the solutions.[97] In an *in vivo* study, an increase in the temperature of the erosive solution resulted in increased erosion depth.[107]

Remineralizing Solution

Delaying brushing after an erosive challenge by storing specimens in artificial saliva appears to significantly[55] increase the resistance to wear of the surface although other studies did not confirm this finding.[108] Storage in human saliva and artificial saliva with different formulations has been used to simulate the clinical situation. Human saliva is capable of forming salivary pellicle which is a protective agent against erosion.[42] It also contains mucins which are lubricants and reduce erosive-abrasive wear.[109] However, the use of human saliva can be difficult since it involves collection from one donor or a pool of donors introducing, a large variation in composition. Human saliva is also sensitive to the storage process and can degrade easily if not stored adequately.[42] One of the main advantages of using artificial saliva is the consistency in the composition and the possibility of being prepared in large amounts. The use of artificial saliva with mucins in its composition is preferable.[109]

MATERIALS AND METHODS

Study Design

This study was conducted according to a factorial 5 × 2 × 2 (surface treatment × abrasive level × time) experimental design. The protective effect of different materials was evaluated against dental erosion and toothbrushing abrasion performed with dentifrices of different abrasive levels over two time periods. Three resin-based materials and one fluoride varnish (Table 1) were used as follow: a pit & fissure sealant, Helioseal® Clear Ivoclair, USA (HS); a dentin sealant, Seal & Protect™, Dentsply, USA (SP) (Figure 1); a resin infiltrant - Icon®,DMG, Germany (IC)(Figure 2); a fluoride varnish, Duraphat® Varnish, Colgate, NY, USA (FV)(Figure 1); and a control (C) with no treatment. The treated surfaces were subjected to erosion and toothbrushing abrasion cycling using hydrochloric acid and slurries containing either low or high abrasives, as defined by the REA/RDA of slurries. The dental substrate (enamel and dentin) was considered as an independent factor and therefore analyzed independently. A total of 80 bovine enamel and dentin specimens were used, with sample size of 8 per group (n=8).

Specimen Preparation

Enamel and dentin slabs (4 mm width × 4 length mm × 2 mm thickness) were cut from bovine incisors using a microtome (Isomet, Buehler, Lake Bluff, IL). After collection and during the preparation process, the teeth were stored in 0.1% thymol solution. The slabs were sequentially ground flat on both sides (top and botton) using silicon carbide grinding papers (Struers RotoPol 31/RotoForce 4 polishing unit, USA). A uniform thickness of approximately 2 mm was created. Slabs were then embedded in acrylic resin (Varidur acrylic system, Buehler, USA) utilizing a custom-made silicon mold, leaving the enamel and dentin surfaces exposed. The resulting blocks (10 mm × 10 mm × 8 mm) containing 1 enamel and 1 dentin specimen were serially ground flat and polished with abrasive discs (500, 1200, 2400 and 4000 grit Al_2O_3 papers; MD-Fuga, Struers Inc., Cleveland, OH) under water cooling. The final polishing was done with a cloth disc with diamond suspension (1 µm; Struers Inc.). Then, the specimen blocks were rinsed with deionized water (DIW), sonicated in detergent solution for three minutes, and selected. Specimens with any cracks or structure defects were discarded. Unplasticised polyvinyl chloride (UPVC) tapes were placed on the polished surface of each specimen, leaving an area of 4×1 mm^2 exposed to subsequent testing. (Figure 3) Specimens were kept in a moist environment until the next step.

Demineralizing Solution

The hydrochloric acid solution of 0.01 M (~pH 2.1) was prepared by mixing 4.23 ml of 22° Bé HCl (UN 1789, Fisher scientific, New Jersey, USA) with ~ 4900 ml of DIW under agitation. A calibrated pH meter was used to determine the pH of the solution, which was adjusted to five liters with DIW. The natural pH was recorded and ranged from 2.11 to 2.13.

Remineralizing Solution

Artificial saliva was prepared by mixing the following reagents (Table 2): $CaCl.H_2O$ (1.065 g); KH_2PO_4 (3.69 g); KCl (5.57 g); NaCl (1.905 g); Tris buffer (60 g) and mucin (11 g) with ~ 4900 ml of deionized water under agitation. The pH was determined using a calibrated pH meter and adjusted to seven with hydrochloric acid. The volume was adjusted to five liters. (Table II)

Abrasive Slurries

Simulated-dentitrifice slurries were prepared, with two levels of abrasivity (low, REA = 4.0±0.8/RDA = 69 and high, REA = 7.1±2.0/RDA = 208±27). The slurries were prepared by mixing the abrasive with an aqueous suspension containing 0.5 percent (w/w) Blanose 7MF carboxymethylcellulose (CMC) and 10 percent (w/w) glycerol.

Lesion Creation

With the objective of creating dental erosion lesions, the specimens were subjected to a short-term acid exposure. The specimens were fully immersed in 0.01 M HCl (~pH 2.3) without agitation, at room temperature, for 30 s (20 ml per block). They were removed, rinsed with deionized water and blotted dry. Tapes were removed from the specimens and a surface area of 2 mm long (X) × 1 mm wide (Y) was scanned with an optical profilometer (Proscan 2000, Scantron, Venture Way, Tauton, UK). The scan covered the treated area and the protected reference surfaces on both sides. The step size was set at 0.01 mm and the number of steps at 200 in the X-axis; and at 0.1 mm and 10, respectively, in the Y-axis. The depth of the treated area was calculated based on the subtraction of the average height of the test area from the average height of the two reference surfaces (tape covered) by using the dedicated software (Proscan Application software v. 2.0.17). These measurements were used for balanced randomization of the specimens into 10 experimental groups (n=8). After baseline measurement, the tape was replaced on the specimen blocks.

Treatments

The sequence of treatment of the groups was done in a randomized manner. The application of the materials followed the manufacturer's instructions and was performed at room temperature. For IC treatment: the specimen surface was dried with air spray for 30 s; Icon-Etch (15% HCl) was applied, left undisturbed for two minutes; the specimen was rinsed with water using an air water syringe for 30 s and air-dried for 30 s; Icon-Dry (ethanol) was applied for 30 s and air-dried for 10 s; Icon-Infiltrant (resin) was applied with the smooth surface applicator tip attached to the syringe and left undisturbed for 3 min; then a cotton roll was used to gently remove excess infiltrant material from the specimen surface before the surface was light-cured for 40 s; a second layer of infiltrant was applied, left undisturbed for 60 s; then a cotton roll was used to gently remove excess infiltrant material from the specimen surface before the surface was light-cured for 40 s. A halogen light curing unit (LCU, Demetron® Optilux 501, Kerr, Orange, CA, USA) with an irradiance of 800 mW/cm^2 was used for all treatments. Output of the curing light was monitored after every 10 specimens using the radiometer built into the curing unit. For HS treatment: the surface was air dried for 30 s; 35 percent phosphoric acid was applied for 30 s and rinsed thoroughly; sealant was applied directly with a disposable cannula, gently dispersed with a microbrush, and left undisturbed for 15 s, then light cured for 20 s. For SP treatment: a drop of water was placed on the specimen, a moist cotton pellet previously saturated with water and blotted on a gauze pad was used to blot excess water from the specimen leaving a moist glistening surface; the sealant was applied to the surface using a microbrush (no scrubbing) leaving a thoroughly wet surface for 20 s; excess sealant was removed by gently air drying for 5 s, then the surface was light cured for 10 s; a second coat of sealant was applied by repeating the steps described above; the oxygen-inhibited layer was removed by wiping the surface with a cotton pellet. For FV treatment: the surface was air dried; a drop of varnish was dispensed to a mixing well, a small brush was used to apply a thin, uniform layer of the material to the surface and the varnish allowed to set for one minute. To assure that the fluoride varnish had the same consistency when applied to all the specimens, a new drop of varnish and a new brush were used for each specimen. All specimens were stored in a moist environment until further use.

Erosion and toothbrushing abrasion cycling (Figure 4)

The daily regimen consisted of four 2-min erosion treatments, four 2-h remineralization treatments in artificial saliva, and two periods of toothbrush abrasion in the toothbrushing machine. The cycling began with the specimens being brushed with their respective assigned slurry for 50 strokes (15 s) using an automated custom-made brushing machine with Oral-B 40 medium stiffness toothbrushes under 150 g of force.[37] (Figure 5) Sixty grams of the slurry were used in each slot of the brushing machine. The specimens were numbered and always placed in the same slot. To avoid contamination of the other specimens by fluoride from the FV and SP groups, the specimens from these groups were brushed with dedicated toothbrushes and slurries. Following brushing, the specimens were thoroughly rinsed with DIW and gently blotted dry, then they were immersed in demineralization solution (Figure 6) without agitation for two minutes, removed, rinsed, blot dried, and stored in artificial saliva stirred at 150 rpm for two hours. After the fourth demin/remin cycle, the specimens were brushed for an additional 50 strokes after which they were stored in artificial saliva overnight. The brushing protocol was run for 5 and 10 days.

Surface Loss Measurement

Surface loss (SL) in µm was measured using an optical profilometer (Figure 7) and analyzed by a dedicated software (Figure 8) as described above after the creation of the lesions as well as after treatment, after 5 and 10 days cycling.

STATISTICAL ANALYSIS

The effects of the surface treatment, dentifrice abrasiveness, and time on the surface loss of enamel and dentin specimens were analyzed using repeated measures analysis of variance (rmANOVA) (Tables III and IV). The repeated measures model allowed different variances by time and correlations between times for each group. Pair-wise comparisons among the groups were made using a Sidak adjustment to control the significance level at 5%.

RESULTS

Dentin results (Figure 9 and 10)

The Effect of the abrasive:

High abrasive had significantly more surface loss than low abrasive at 10 days ($p=0.0280$), but no other significant differences were found.

The effect of time:

For C: 5 days had significantly more surface loss than demin ($p=0.0324$) and treatment ($p=0.0360$), and 10 days had significantly more surface loss than demin ($p=0.0001$) and treatment ($p=0.0001$), but demin and treatment were not significantly different from each other ($p=0.23$) and 5 days and 10 days were not significantly different from each other ($p=0.31$).

For FV 10 days had significantly more surface loss than demin, treatment, and 5 days ($p<0.0001$); demin had more surface loss than treatment ($p<0.0001$) and 5 days ($p=0.0004$), but treatment and 5 days were not significantly different from each other ($p=0.48$).

For HS, demin had significantly more surface loss than treatment, 5 days, and 10 days ($p<0.0001$); day 5 had less surface gain than treatment ($p=0.0022$) and 10 days ($p=0.0003$); and treatment had less surface gain than 10 days ($p=0.0051$).

For IC, treatment ($p=0.0177$), 5 days ($p=0.0003$), and 10 days ($p=0.0001$) had more surface loss than demin; and 10 days had more surface loss than treatment ($p=0.0045$); but 5 days was not significantly different from treatment ($p=0.53$) or 10 days ($p=0.09$).

For SP, demin had significantly more surface loss than treatment, 5 days, and 10 days ($p \leq 0.0001$); 10 days had more surface gain than 5 days ($p=0.0197$); but treatment was not significantly different from 5 days ($p=0.53$) or 10 days ($p=0.76$).

The effect of treatment

At demin: No differences were found between treatments at demin ($p=0.66$).

At treatment: FV, HS, and SP had significantly more surface gain than C and IC ($p<0.0001$), and IC had more surface loss than C ($p=0.0287$), but there were no significant differences among FV, HS, and SP ($p>0.08$).

At 5 days: FV, HS, and SP had significantly more surface gain than C and IC ($p \leq 0.0001$), but there were no significant differences between C and IC ($p=1.00$) or among FV, HS, and SP ($p>0.10$).

At 10 days: HS and SP had significantly more surface gain than C, IC, and FV ($p<0.0001$), and FV had significantly less surface loss than C for low abrasive ($p=0.0009$), but there were no significant differences between C and IC ($p=1.00$), IC and FV ($p=0.10$), C and FV for high abrasive ($p=0.78$), or HS and SP ($p=0.07$).

Enamel results (Figure 11 and 12)

The effect of the abrasive

High abrasive had significantly more surface loss than low abrasive for C at 5 days ($p=0.0117$) and at 10 days ($p=0.0162$) and for FV after treatment ($p=0.0441$), but no other significant differences were found.

The effect of time

For: C 10 days had significantly more surface loss than demin, treatment, and 5 days ($p<0.0001$); and 5 days had more surface loss than demin and treatment ($p<0.0001$); but demin and treatment were not significantly different from each other ($p=0.90$).

For FV, 10 days had significantly more surface loss than treatment ($p<0.0001$) and 5 days ($p=0.0010$); treatment had significantly more surface gain than demin and 5 days ($p\leq0.0001$); and 5 days had significantly more surface gain than demin ($p=0.0055$); but demin and 10 days were not significantly different from each other ($p=0.16$).

For HS, demin had significantly more surface loss than treatment, 5 days, and 10 days ($p<0.0001$); 10 days had significantly more surface gain than 5 days ($p<0.0001$); but treatment was not significantly different from 5 days ($p=0.86$) or 10 days ($p=0.93$).

For IC, 5 days ($p=0.0013$) and 10 days ($p=0.0007$) had more surface loss than demin; 5 days ($p=0.0001$) and 10 days ($p<0.0001$) had more surface loss than treatment; and 10 days had more surface loss than 5 days ($p=0.0003$); but demin and treatment were not significantly different from each other ($p=0.47$).

For SP, treatment had more surface gain than demin ($p=0.0004$) and 5 days ($p=0.0264$); demin was not significantly different from 5 days ($p=0.0504$) or 10 days ($p=0.92$), and treatment was not significantly different from 10 days ($p=0.41$).

The effect of treatment

At demin: No differences were found between treatments at demin ($p=0.99$).

At treatment: FV, HS, and SP had significantly more surface gain than C ($p<0.0006$) and IC ($p<0.0001$), and FV and HS had more surface gain than SP for low abrasive ($p\leq0.01$), but there were no significant differences between C and IC ($p=0.55$), FV e HS ($p=0.77$), or FV and SP for high abrasive ($p=0.91$), or HS and SP for high abrasive ($p=0.38$).

At 5 days: FV, HS and SP had significantly more surface gain than C ($p<0.0008$) and IC ($p<0.0003$), and HS had more surface gain than SP ($p=0.0001$), but there were no significant differences between C and IC ($p=1.00$), FV and HS ($p=0.06$), or FV and SP ($p=0.34$).

At 10 days: HS had significantly more surface gain than all other treatments ($p<0.0001$), and FV and SP had significantly less surface loss than C ($p<0.0013$) and IC ($p<0.0040$), but there were no significant differences between C and IC ($p=1.00$) or FV and SP ($p=0.48$).

FIGURES AND TABLES

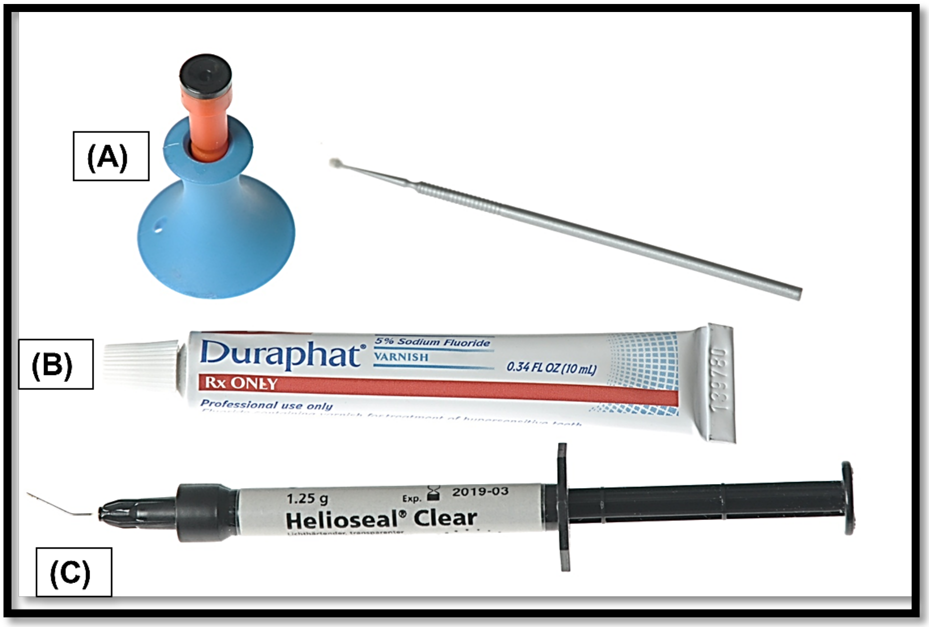

Figure 1. Treatment materials (A) Seal and Protect (B) Duraphat (C) Helioseal Clear

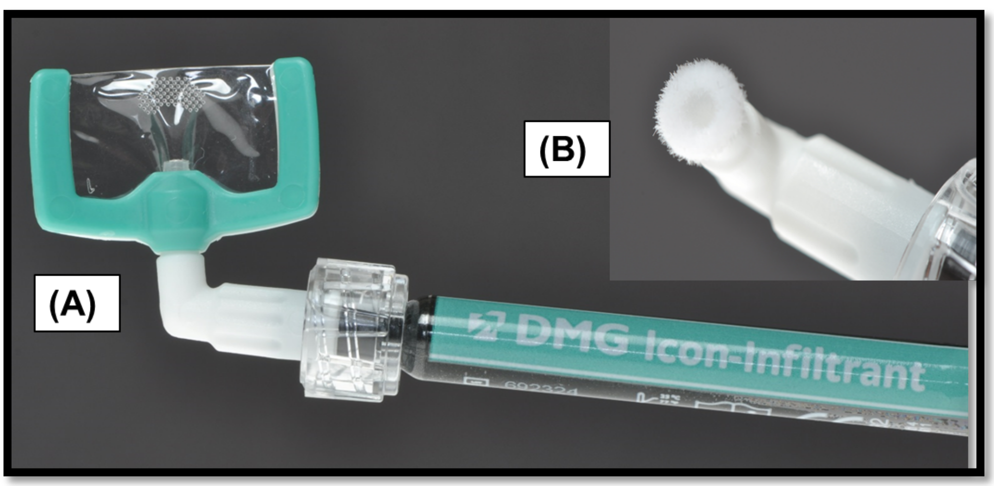

Figure 2. (A) Icon resin infiltrant (B) Smooth surface applicator

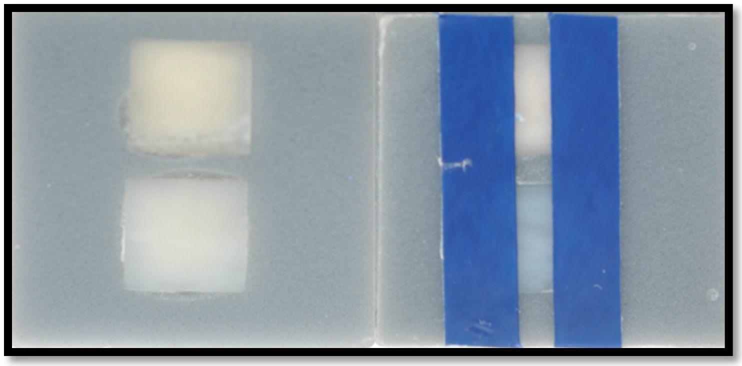

Figure 3. Enamel and dentin slab embedded in resin; tape was placed leaving a treatment window

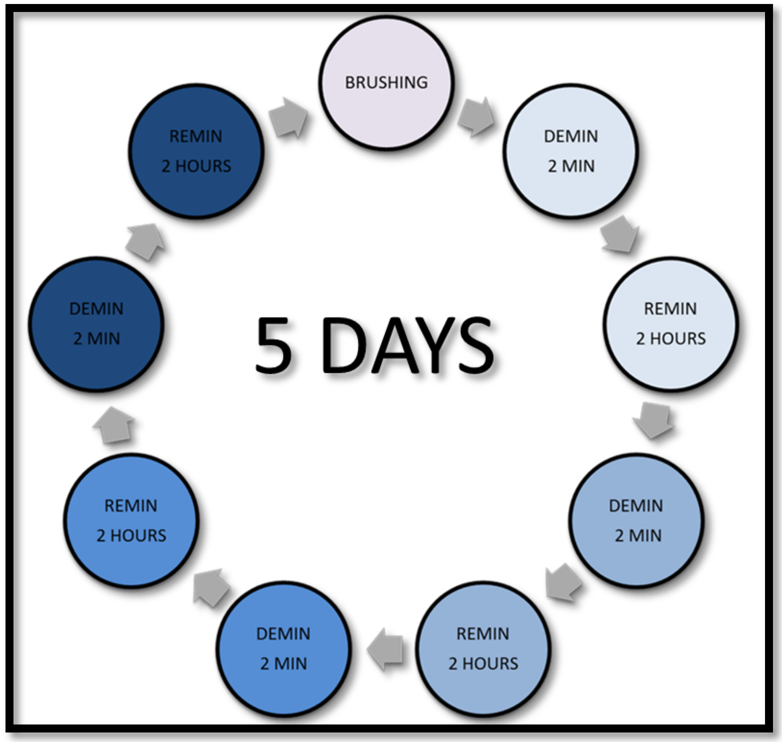

Figure 4. Erosion- abrasion daily cycle

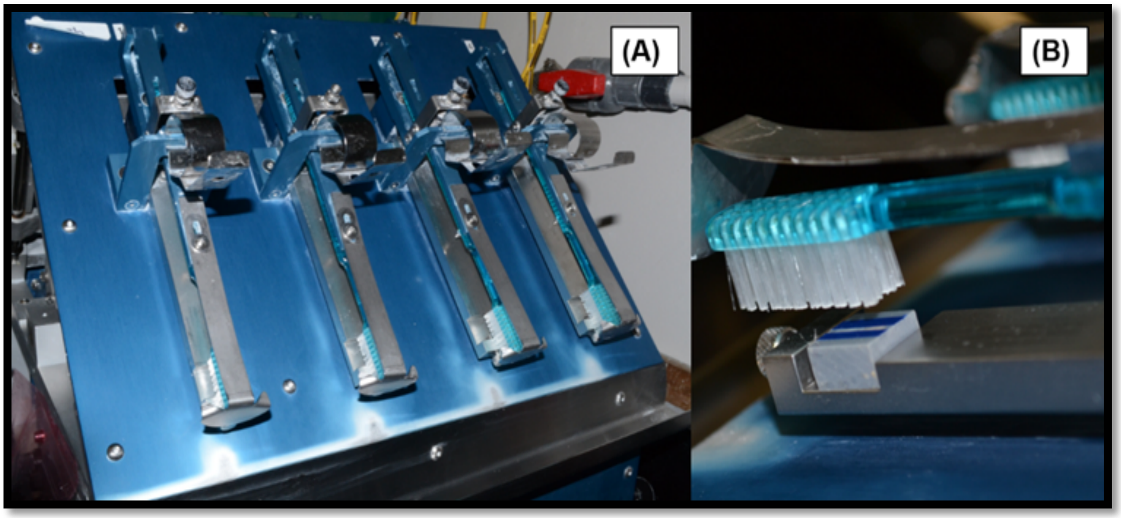

Figure 5. (A) Automated brushing machine (B) Positioning of the specimen

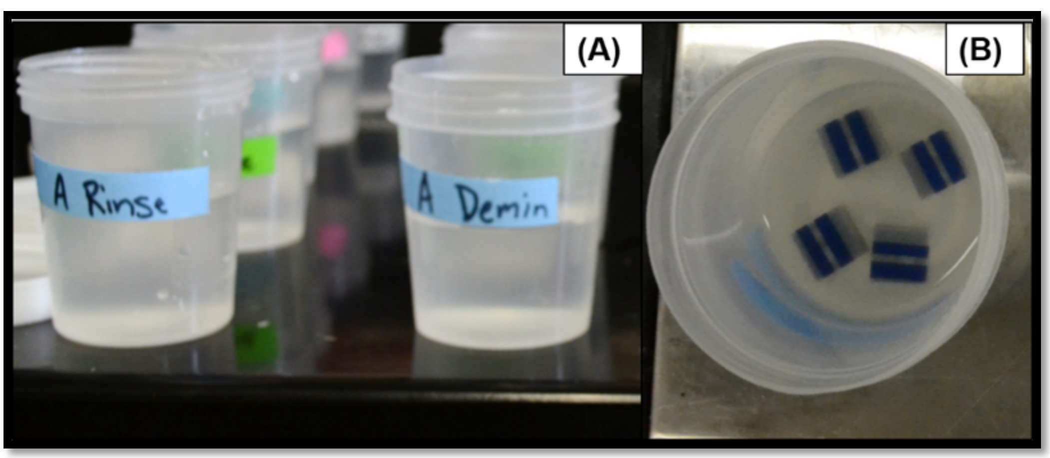

Figure 6. (A) Demin/remin setup (B) Erosive challenge

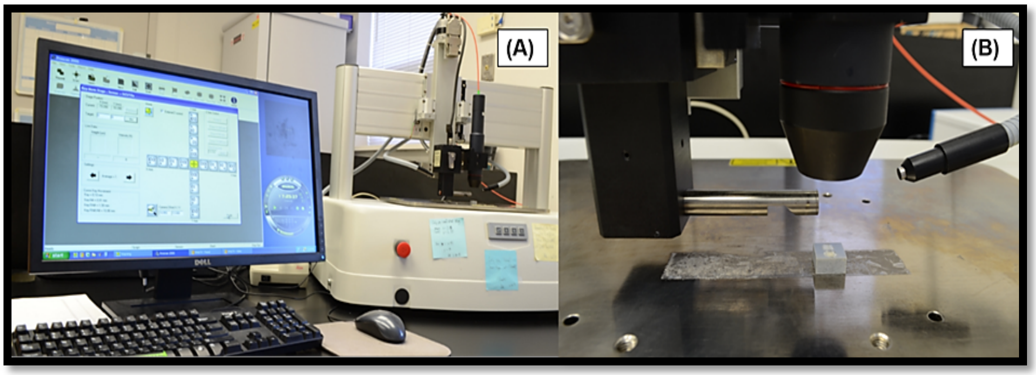

Figure 7. (A) Optical profilometer (B) Positioning of the specimen

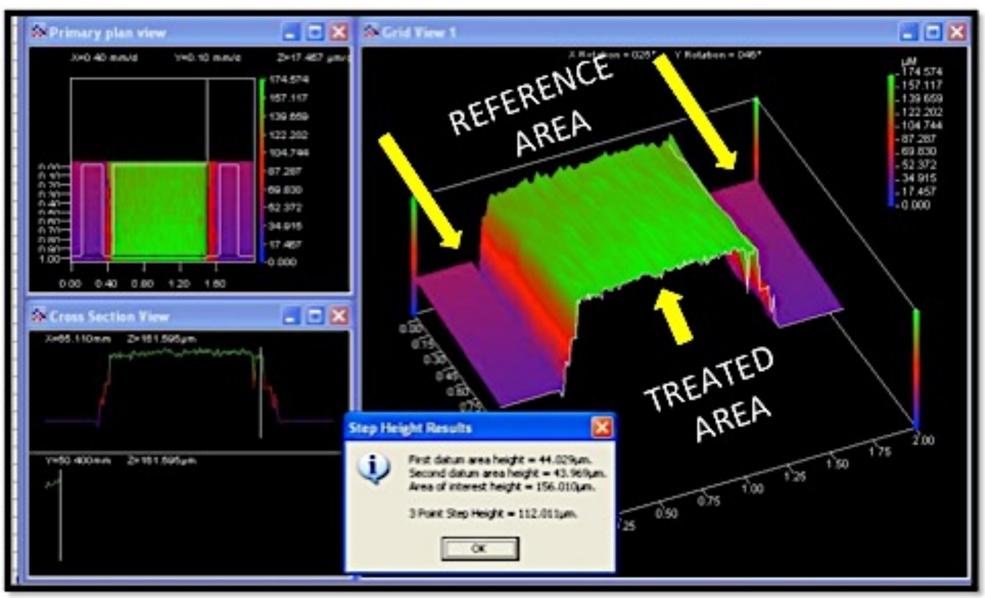

Figure 8. Output screen from the optical profilometer analysis software

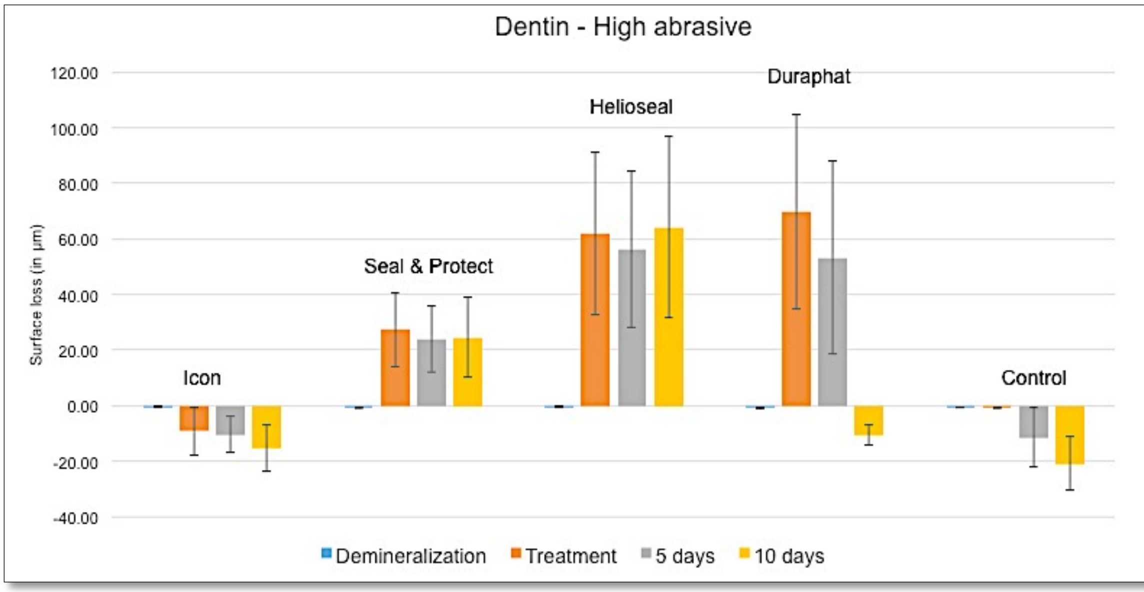

Figure 9. Bar graph of surface loss on dentin for different materials brushed with high abrasive

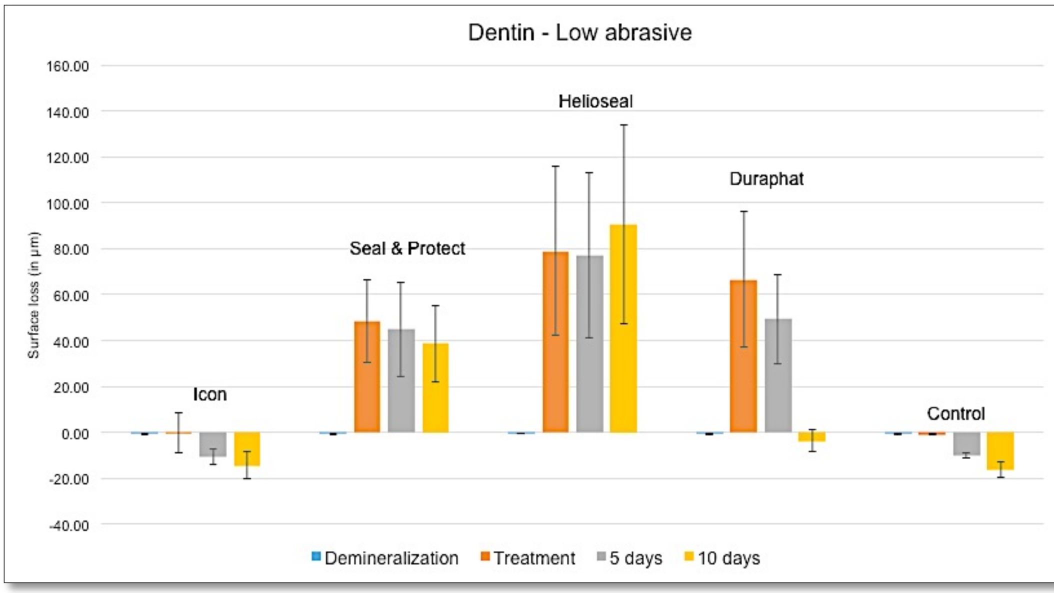

Figure 10. Bar graph of surface loss on dentin for different materials brushed with low abrasives

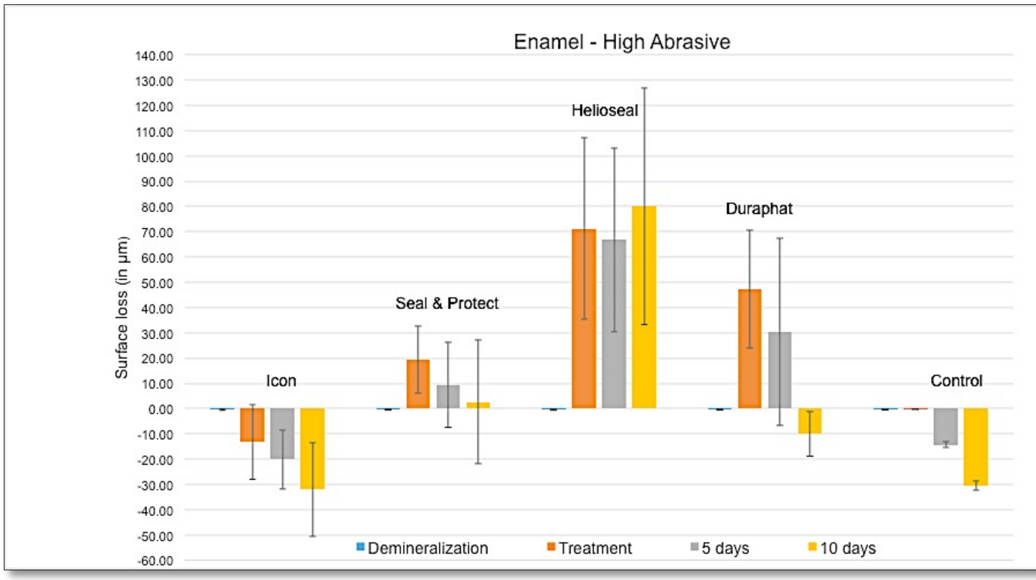

Figure 11. Bar graph of surface loss on enamel for different materials brushed with high abrasives

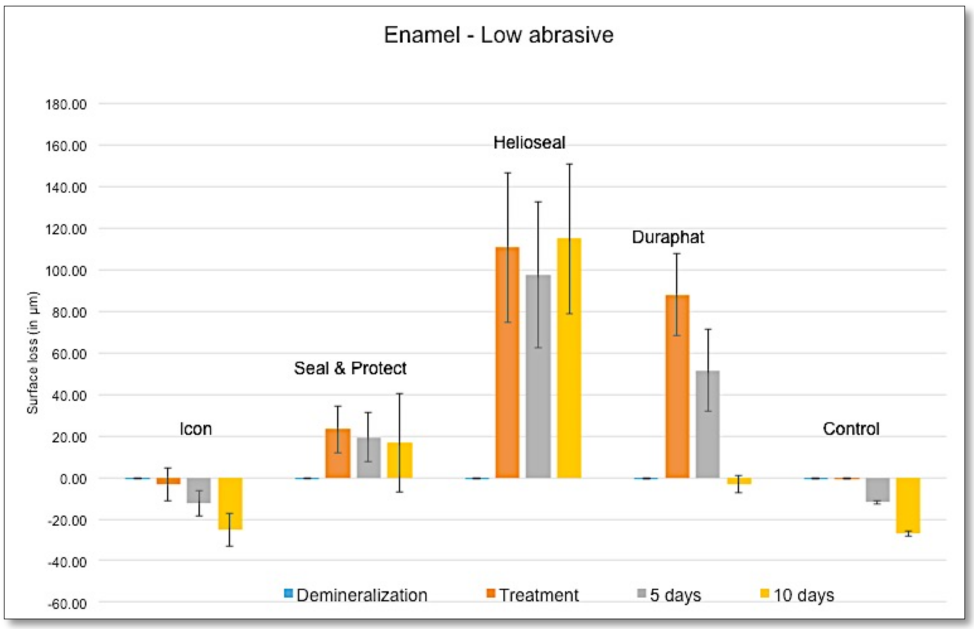

Figure 12. Bar graph of surface loss on enamel for different materials brushed with low abrasives

TABLE I

Composition of Treatment Materials

MATERIAL	COMPOSITION	MANUFACTURER
ICON (infiltrant)	Icon-etch: 15%hydrochloric acid, pyrogenic silicic acid, surface-active substances, Icon-dry: 99% ethanol; Icon-infiltrant: TEGDMA, initiators, and additives	DMG, Germany
SEAL&PROTECT (sealant for exposed dentin)	Di and trimethacrylate resins; PENTA (dipentaerythritol penta acrylate monophosphate); nano fillers (amorphous silicone dioxide); photoinitiators; stabilizers; cetylamine hydrofluoride; triclosan; acetone	Ivoclar, USA
HELIOSEAL (pit and fissure sealant)	Bis-GMA, TEGDMA, and additives	Dentsply, USA
DURAPHAT (fluoride varnish)	Colophonium; ethanol; sodium fluoride; saccharin; isoamyl acetate	Colgate, USA

TABLE II

Composition of Artificial Saliva

REAGENTS	QUANTITY g/L
$CaCl_{2}*2H_{2}O$	0.213
KH_2PO4	0.738
KCl	1.114
NaCl	0.381
Tris buffer	12
Mucin	2.2

TABLE III

ANOVA table for dentin

Substrate	Effect	Num DF	Den DF	F Value	p-value
Dentin	Treatment	4	25.81	77.52	<.0001
	Abrasive	1	28.09	4.90	0.0351
	Treatment*Abrasive	4	25.81	2.00	0.1240
	Time	3	18.45	68.71	<.0001
	Treatment*Time	12	19.78	30.00	<.0001
	Abrasive*Time	3	18.45	3.30	0.0435
	Treatment*Abrasive*Time	12	19.78	2.55	0.0317

TABLE IV

ANOVA table for enamel

Substrate	Effect	Num DF	Den DF	F Value	p-value
Enamel	Treatment	4	23.09	71.78	<.0001
	Abrasive	1	29.10	9.59	0.0043
	Treatment*Abrasive	4	23.09	2.42	0.0777
	Time	3	22.69	161.58	<.0001
	Treatment*Time	12	25.65	77.26	<.0001
	Abrasive*Time	3	22.69	5.52	0.0054
	Treatment*Abrasive*Time	12	25.65	5.41	0.0002

TABLE V

Least squares means and standard error for dentin surface loss comparing treatment, abrasive and time

Dentin		Demin	Treatment	5d	10d
Material	Abrasive				
IC	High	-0.60 (0.10) A a	-9.90 (2.68) C c	-10.57 (2.27) B bc	-15.43 (2.86) * B b
SP	High	-0.65 (0.08) A a	27.08 (4.67) A bc	25.47 (4.22) A c	25.76 (4.85) A b
HS	High	-0.60 (0.09) A a	61.94 (10.33) A c	56.53 (9.93) A b	66.23 (11.11) A d
FV	High	-0.67 (0.10) A b	69.70 (12.40) A c	53.28 (12.31) A c	-10.43 (1.29) B a
C	High	-0.59 (0.03) A b	-0.69 (0.07) B b	-11.45 (3.81) B a	-20.89 (3.41) * B a
IC	Low	-0.68 (0.08) A a	-6.09 (1.84) C c	-10.85 (1.14) B bc	-14.12 (2.29) *BC b
SP	Low	-0.73 (0.08) A a	48.52 (6.49) A bc	46.42 (6.67) A c	55.13 (7.90) A b
HS	Low	-0.56 (0.06) A a	79.04 (12.95) A c	77.24 (12.73) A b	90.65 (15.30) A d
FV	Low	-0.65 (0.08) A b	66.69 (10.49) A c	49.33 (6.92) A c	-5.28 (0.80) B a
C	Low	-0.64 (0.08) A b	-0.83 (0.08) B b	-9.93 (0.41) B a	-16.21 (1.27) * C a

Different capital letters represent significant differences within column
Different lower case letters represent significant differences within row
Mean values followed by * are different in comparison with the abrasives within the materials

TABLE VI

Least squares means and standard error for enamel surface loss comparing treatment, abrasive and time

Enamel Material	Enamel Abrasive	Post-demin	Post-treatment	5d	10d
IC	High	-0.44 (0.09) A c	-8.81 (4.19) * B c	-20.04 (4.16) C b	-32.01 (6.59) C a
SP	High	-0.41 (0.06) A b	22.75 (5.59) * A a	13.67 (7.12) B b	7.57 (10.33) * A ba
HS	High	-0.43 (0.08) A a	71.27 (12.65) A bc	66.82 (12.88) A c	81.17 (16.05) A b
FV	High	-0.41 (0.06) A a	47.24 (8.27) A b	30.35 (13.08) AB c	-9.96 (3.14) B a
C	High	-0.39 (0.06) A c	-0.28 (0.06) * B c	-14.30 (0.41) C b	-30.51 (0.66) C a
IC	Low	-0.41 (0.07) A c	-4.86 (2.20) * C c	-12.25 (2.14) C b	-24.16 (2.63) C a
SP	Low	-0.39 (0.07) A b	23.18 (4.05) * B a	19.26 (4.19) B b	16.56 (8.36) * B ba
HS	Low	-0.41 (0.08) A a	110.64 (12.73) A bc	97.53 (12.41) A c	119.63 (15.15) A b
FV	Low	-0.43 (0.09) A a	87.94 (7.04) A b	51.52 (7.02) AB c	-3.09 (1.51) B a
C	Low	-0.43 (0.09) A c	-0.41 (0.10) * C c	-12.02 (0.27) C b	-26.91 (0.50) C a

Different capital letters represent significant differences within column
Different lower case letters represent significant differences within row
Mean values followed by * are different in comparison with the abrasives within the materials

TABLE VII

Complete data set for dentin

Treatment	Abrasive	Time	N	Mean	SD	SE	95% CI for Mean		Min	Max
Control	high	Demin	8	-0.59	0.08	0.03	-0.66	-0.53	-0.68	-0.45
		Treatment	8	-0.69	0.20	0.07	-0.86	-0.53	-0.89	-0.35
		5-day	8	-11.45	10.78	3.81	-20.46	-2.44	-26.40	11.40
		10-day	8	-20.89	9.64	3.41	-28.95	-12.83	-39.93	-13.40
	low	Demin	8	-0.64	0.22	0.08	-0.82	-0.46	-0.98	-0.32
		Treatment	8	-0.83	0.23	0.08	-1.02	-0.64	-1.08	-0.44
		5-day	8	-9.93	1.15	0.41	-10.89	-8.96	-11.45	-7.75
		10-day	8	-16.21	3.60	1.27	-19.22	-13.20	-20.97	-10.62
Duraphat	high	Demin	8	-0.67	0.27	0.10	-0.90	-0.45	-1.12	-0.36
		Treatment	8	69.70	35.07	12.40	40.38	99.02	30.77	140.74
		5-day	8	53.28	34.82	12.31	24.16	82.39	-6.54	98.96
		10-day	8	-10.43	3.66	1.29	-13.49	-7.37	-16.09	-6.86
	low	Demin	8	-0.65	0.22	0.08	-0.83	-0.47	-1.14	-0.39
		Treatment	8	66.69	29.67	10.49	41.88	91.49	35.68	121.29
		5-day	8	49.33	19.56	6.92	32.97	65.68	29.96	82.80
		10-day	8	-5.28	2.27	0.80	-7.17	-3.38	-8.13	-1.46
Helioseal	high	Demin	8	-0.60	0.25	0.09	-0.81	-0.39	-1.11	-0.37
		Treatment	8	61.94	29.23	10.33	37.50	86.38	25.33	102.08
		5-day	8	56.53	28.09	9.93	33.05	80.01	21.78	94.24
		10-day	8	66.23	31.42	11.11	39.96	92.50	30.76	108.06
	low	Demin	8	-0.56	0.16	0.06	-0.70	-0.43	-0.78	-0.29
		Treatment	8	79.04	36.62	12.95	48.42	109.65	26.85	120.49
		5-day	8	77.24	36.00	12.73	47.14	107.33	24.41	118.12
		10-day	8	90.65	43.29	15.30	54.46	126.84	26.03	136.92
Icon	high	Demin	8	-0.60	0.27	0.10	-0.83	-0.38	-1.03	-0.25
		Treatment	8	-9.90	7.57	2.68	-16.23	-3.56	-19.61	1.88
		5-day	8	-10.57	6.41	2.27	-15.93	-5.21	-20.33	-2.30
		10-day	8	-15.43	8.08	2.86	-22.18	-8.67	-27.10	-5.32
	low	Demin	8	-0.68	0.23	0.08	-0.87	-0.49	-1.00	-0.33
		Treatment	8	-6.09	5.20	1.84	-10.44	-1.74	-12.47	0.59
		5-day	8	-10.85	3.23	1.14	-13.55	-8.14	-15.89	-6.39
		10-day	8	-14.12	6.49	2.29	-19.55	-8.70	-20.41	-2.10
S&P	high	Demin	8	-0.65	0.23	0.08	-0.85	-0.46	-0.97	-0.35
		Treatment	8	27.08	13.21	4.67	16.04	38.12	8.85	43.64
		5-day	8	25.47	11.95	4.22	15.48	35.46	8.22	43.51
		10-day	8	25.76	13.73	4.85	14.29	37.24	5.08	45.68
	low	Demin	8	-0.73	0.22	0.08	-0.91	-0.55	-0.98	-0.38
		Treatment	8	48.52	18.35	6.49	33.18	63.86	29.08	89.61
		5-day	8	46.42	18.85	6.67	30.66	62.18	26.70	88.37
		10-day	8	55.13	22.36	7.90	36.44	73.82	32.60	107.02

TABLE VIII

Complete data set for enamel

Treatment	Abrasive	Time	N	Mean	SD	SE	95% CI for Mean		Min	Max
Control	high	Demin	8	-0.39	0.17	0.06	-0.53	-0.25	-0.66	-0.15
		Treatment	8	-0.28	0.18	0.06	-0.42	-0.13	-0.52	-0.04
		5-day	8	-14.30	1.17	0.41	-15.28	-13.32	-15.94	-12.75
		10-day	8	-30.51	1.88	0.66	-32.08	-28.94	-32.51	-27.10
	low	Demin	8	-0.43	0.24	0.09	-0.63	-0.23	-0.92	-0.17
		Treatment	8	-0.41	0.29	0.10	-0.66	-0.17	-0.89	-0.05
		5-day	8	-12.02	0.78	0.27	-12.67	-11.37	-13.18	-10.82
		10-day	8	-26.91	1.42	0.50	-28.09	-25.72	-28.77	-24.67
Duraphat	high	Demin	8	-0.41	0.17	0.06	-0.55	-0.27	-0.71	-0.21
		Treatment	8	47.24	23.40	8.27	27.67	66.80	21.91	97.08
		5-day	8	30.35	37.00	13.08	-0.58	61.29	-6.47	111.11
		10-day	8	-9.96	8.89	3.14	-17.39	-2.52	-27.56	-0.71
	low	Demin	8	-0.43	0.25	0.09	-0.64	-0.23	-0.93	-0.10
		Treatment	8	87.94	19.90	7.04	71.30	104.58	62.76	119.27
		5-day	8	51.52	19.85	7.02	34.93	68.12	18.25	84.06
		10-day	8	-3.09	4.26	1.51	-6.65	0.47	-11.02	3.94
Helioseal	high	Demin	8	-0.43	0.23	0.08	-0.63	-0.24	-0.88	-0.14
		Treatment	8	71.27	35.78	12.65	41.36	101.18	25.05	124.65
		5-day	8	66.82	36.43	12.88	36.36	97.28	23.40	125.55
		10-day	8	81.17	45.38	16.05	43.23	119.11	28.17	154.13
	low	Demin	8	-0.41	0.21	0.08	-0.59	-0.23	-0.83	-0.16
		Treatment	8	110.64	36.00	12.73	80.54	140.74	57.32	157.89
		5-day	8	97.53	35.11	12.41	68.18	126.88	48.70	153.78
		10-day	8	119.63	42.85	15.15	83.81	155.45	60.28	185.45
Icon	high	Demin	8	-0.44	0.24	0.09	-0.64	-0.23	-0.90	-0.18
		Treatment	8	-8.81	11.84	4.19	-18.71	1.09	-15.62	19.93
		5-day	8	-20.04	11.76	4.16	-29.87	-10.21	-29.78	5.02
		10-day	8	-32.01	18.63	6.59	-47.59	-16.44	-46.59	10.18
	low	Demin	8	-0.41	0.19	0.07	-0.57	-0.25	-0.64	-0.10
		Treatment	8	-4.86	6.21	2.20	-10.06	0.33	-15.77	2.08
		5-day	8	-12.25	6.04	2.14	-17.30	-7.20	-23.23	-5.98
		10-day	8	-24.16	7.44	2.63	-30.38	-17.94	-36.87	-18.07
S&P	high	Demin	8	-0.41	0.18	0.06	-0.56	-0.26	-0.66	-0.15
		Treatment	8	22.75	15.80	5.59	9.55	35.96	0.71	44.14
		5-day	8	13.67	20.13	7.12	-3.17	30.50	-12.79	42.78
		10-day	8	7.57	29.21	10.33	-16.85	31.99	-32.88	48.03
	low	Demin	8	-0.39	0.18	0.07	-0.54	-0.23	-0.66	-0.10
		Treatment	8	23.18	11.45	4.05	13.60	32.75	10.73	45.68
		5-day	8	19.26	11.84	4.19	9.36	29.16	7.71	41.28
		10-day	8	16.56	23.63	8.36	-3.20	36.31	-12.40	51.42

DISCUSSION

Experimental model

This study investigated the efficacy of resin-based materials and fluoride varnish on the protection of dental surfaces against dental erosion, when submitted to different abrasive challenges. Resin-based materials constitute a mechanical barrier preventing erosive acids from contacting and, eventually, wearing-away enamel and dentin. This treatment option targets patients at extremely high risk for dental erosion, such as those suffering from intrinsic erosion, where conventional preventive treatments (fluoridated toothpastes, gels and mouthrinses) present limited or no efficacy.[34, 38, 39] In order to mimic those clinical conditions, this experimental model used included four erosive challenges per day with 0.01 M HCl at natural pH (~pH 2.1),[36,40-42] for 2 min each time. This represents a conservative exposure to the acid, since pH telemetry in patients with GERD found the total time of erosion to be between 4.3 and 60 min per day.[43] Hydrochloric acid is a strong acid and the main component of the gastric juice. The gastric juice is also contains proteolytic enzymes such as pepsin, which can further degrade the organic matrix of the exposed dentin,[44] affecting fluoride efficacy.[45] However, to avoid the use of a complex erosive solution and to compare our study to in vitro studies in this area, this was not simulated presently. Therefore, this should be taken into account when interpreting the results for dentin.

Toothbrushing simulation was performed twice a day, for 15 s[37] (50 double brushing strokes). This represents a total brushing time of 1.5 min, assuming a brushing time of 15 s, for each sextant. Standard toothbrushes of medium stiffness were used with a load of 150 g. The tested abrasive suspensions simulated different degrees of abrasivity of toothpastes, including low and high. The abrasive levels were previously determined in the suspensions using a standard test (RDA, radioactive dentin abrasivity, ISO11609). The low-abrasivity suspension presented a mean value (standard-deviation) of REA 4.0 (0.8) / RDA 69 (7.0), while the high one of REA 7.1 (2.0) / RDA 208 (27.0). These values may represent the extremes of abrasivity commonly observed in commercially available toothpastes, from anti-sensitivity (usually less abrasive) to whitening (usually more abrasive) formulations.[46] This abrasive challenge focused only on toothbrushing, and did not account for other abrasive forces, such as those caused by dental attrition and food and soft tissue and food contacts.

Resin-based materials tested

In this study three resin based materials and one fluoride varnish were applied to softened enamel and dentin. IC is a low-viscosity resin indicated to prevent the progression of incipient enamel lesions on smooth and interproximal surfaces. Although a previous study[36] investigated the effect of IC to prevent erosion, there are no reports on its efficacy preventing dental erosion-abrasion. Moreover, this is the first study to investigate its application on dentin under these conditions. HS is a pit and fissure sealant and has been shown to protect against erosive tooth wear on specimens positioned at the palatal surface of anterior incisors, clinically. The HS-treated patients presented a mean surface wear of 30 µm compared to 140 µm from the control group, at 20 month.[33] SP is a dentin-desensitizing agent and has been used as an alternative approach to control erosion in enamel and dentin.

Surface height change (loss/deposition) after lesion creation and treatment

Erosive lesions were created at the beginning of the experiment, in order to simulate patients already suffering from intrinsic erosion. This created a more realistic condition for the dental substrate to be treated with the surface coating materials. Even though the exposure of the dental substrate to the acid was limited to 2 min, relatively small but measurable surface loss was observed, with numerically higher values for dentin. No difference was observed among groups within each substrate, which was expected to occur, since no treatment effects (coating material and dentifrice abrasive) had happened at that point. This verified the validity of the stratified randomization procedure performed, for balanced distribution of specimens into test groups. After application of the testing materials, there was significant gain on the height of the experimental surface, due to the expected deposition of the resin materials SP and HS, and fluoride varnish (FV). The deposition was generally similar on these three groups on both dental substrates, although HS showed numerically higher mean values. These were significantly higher than the non-treated negative control group and IC. Although differences in deposition should not have been observed within each material after the application, HS and FV showed some significant changes for enamel (comparison among groups associated to the high and to the low abrasives). This indicates that the thickness of these materials is highly variable, which can be confirmed also by the relatively higher standard deviation values observed in these groups (Tables V and VI). It is important to take this information into consideration for clinical application, as the procedures were standardized (flat specimens, easy access for the material, adequate illumination and moisture), which may not be necessarily true under real conditions. The resin infiltrant (IC) showed similar surface height values as the control group, for enamel; while significantly more surface loss than the control group, for dentin. This was probably observed because of the acid etching-step, before the application of the resin. It is possible that the enamel substrate that was etched-away was restored by the resin application, hence the net result similar to the control group (no meaningful changes in substrate height). However, the dentin loss may have been greater, as dentin is more susceptible to demineralization than enamel, [47] therefore, this relatively thin resin did not totally replace the thickness of dentin that was lost. Consequently, significantly higher surface loss values were observed.

Material/dental substrate loss at day 5, Enamel and Dentin

After 5 days of erosion-toothbrushing abrasion cycling, significantly higher surface loss was observed for the negative control group as expected, validating the ability of the cycling model to promote the development of erosive-abrasive lesions on enamel. HS, SP and FV showed some loss of the material, however they were still present, which confirmed their ability to protect enamel, even after the 5 cycling days. HS presented significantly higher deposition than SP, while FV showed intermediary values. The retention of the FV was somewhat surprising, giving the mechanical properties of this material compared to the resin-based ones. However, it may indicate that in the short-term (a few days) it may be a relevant alternative. Resin based materials will function as a barrier to erosion and abrasion wear, and depending on the filler content and other mechanical properties they will protect the hard tissue for limited periods of time. IC showed significant surface loss, at the same rate observed for the control group. This indicates that either there was little/no infiltration of the material in eroded enamel or the infiltrated eroded enamel provided no additional resistance to subsequent erosive-abrasive challenges. The limited infiltration of IC can be explained, as erosive lesions are typically shallower than caries lesion, the intended target of this material. Therefore, a very thin or no-infiltrated layer may have been created. Further analysis of the dental substrate would have to be done to confirm this speculation. Also, the resin materials, although effective at blocking acid permeation into the dental tissue, may not present adequate mechanical properties due to the lack of fillers to resist toothbrushing abrasive forces, as simulated in this study.

The abrasive level of the simulated toothpaste showed to be relevant only for the control group, with more surface loss to the more abrasive slurry; while no significant effects were observed for the other materials. This result corroborates observations for the high abrasive toothpastes in previous studies using a similar model.[48]

On dentin, numerical trends similar to those for enamel were observed, with marked progression observed in the control group and IC. However, no clear differences were observed between HS, SP and FV. Interestingly, no abrasive effect was observed for dentin even in the control group, despite the presence of expected numerical trend. This result seems to contradict the literature, as more abrasive toothpastes have generally shown to cause more dentin surface loss.[48]

Material/dental substrate loss at day 10, Enamel and Dentin

After 10 days of cycling, there was an increase in surface loss for C, which doubled the loss observed at day 5, confirming the ability of the model to simulate progression of the lesions. Deposits of HS and SP were still present, although significant loss was observed for SP in the enamel substrate when brushed with high abrasive suspension. This confirmed their ability to protect tooth structure against toothbrushing wear and also show the influence of the abrasive level on the progression of the erosive-abrasive lesions. Interestingly, the HS group presented a significant increase in the surface height compared to day 5. This unexpected result may be explained by the presence of Bis-GMA/TEGDMA monomers in its composition. These monomers, when in aqueous environment, may suffer water absorption resulting in an increase of the material volume.[49] FV was completely removed after 10 days but some protective effect was still observed when compared to C. This result is in agreement with a previous *in vitro* study,[50] where the same FV (Duraphat) was tested. However, in that study the FV was mechanically removed after the application, which does not represent the clinical reality. In the present study we kept the varnish, as that would be relevant for the test of the toothbrushing effects. IC showed significant loss, similar to C, confirming that in this study model no beneficial effect of an infiltrant was observed in the protection against erosion and abrasion.

The abrasive level of the simulated toothpaste had a significant effect on the surface loss for the control group in enamel, whereas no influence was observed for the other material. The high abrasive, however, significantly affected dentin regardless of the surface treatment at 10 days. This result is in agreement with studies[51] that have investigated the effect of toothpaste abrasivity on the wear of eroded dentin.

On enamel, a significantly higher surface loss was observed mainly for the IC and C. This is in agreement with studies that showed that progression of tooth wear is different for enamel and dentin. This may be explained by the fact that, while the dissolution of the enamel occurs layer by layer, in dentin after the mineral layer is removed the collagen is exposed acting as a barrier buffering the acids and decreasing mineral dissolution.[52]

SUMMARY AND CONCLUSIONS

The objectives of this *in vitro* study were: to evaluate the protective effect provided by three resin-based materials (pit & fissure sealant - Helioseal® Clear Ivoclair, USA; dentin sealant - Seal & Protect™,Dentsply, USA, and resin infiltrant - Icon®,DMG, Germany), one fluoride varnish (Duraphat® Varnish, Colgate, NY, USA) and no treatment (control group) against dental erosion and tooth brushing abrasion on enamel and dentin. We also evaluated the influence of the abrasive level of the dentifrice and time on the protective effect of these materials.

Under the limitations imposed by the in vitro nature of this study, we concluded that:

1. The treatments tested presented different protective effects against erosion-toothbrushing abrasion challenges, with HS being the most effective material, with SP showing relatively lower protection. FV was also able to protect the dental substrate, although this was limited to the initial 5 days of testing. No benefit was observed for the resin infiltrant IC.

2. The simulated high-abrasivity dentifrice slurry generally led to numerically higher substrate (dental and/or material) loss than the low-abrasivity one; however, this was significant only in specific comparisons of the control group.

3. The simulated erosion-abrasion times tested (5 and 10 days) showed that FV can persist on the dental surfaces and, therefore, present significant protection against erosion-abrasion for short periods of time (up to 5 days).

4. The protective effect of the tested materials was generally similar in both enamel and dentin, although enamel seemed to be more prone to erosion-abrasion, in the experimental model used.

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ABSTRACT

EFFICACY OF RESIN-BASED MATERIALS

AGAINST EROSIVE-ABRASIVE

WEAR IN VITRO

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Background: Increasing prevalence of dental erosion has been observed in many countries, in both children and adults. This condition is often associated with softening of the dental surface by acid exposure, which may lead to severe and irreversible damage. The use of fluoride, pit and fissure sealants, dental adhesives and more recently a resin infiltrant has been suggested to manage dental erosion. **Objective:** To compare the protective effect of a resin infiltrant and other resin-based materials against dental erosion/toothbrushing abrasion in vitro. **Materials and methods**: Bovine enamel and dentin slabs were prepared, embedded, flattened and polished. Dental erosion lesions were created using 0.01 M of hydrochloric acid (pH 2.3 for 30 sec) and treated with resin-based materials (HS: Helioseal pit and fissure sealant; SP: Seal and Protect dentin

sealant, and IC: Icon resin infiltrant) or fluoride varnish (FV: Duraphat). A no-treatment group represented the negative control (C). The specimens were subjected to an erosion-abrasion cycling model for a total of 10 days. Each cycling day consisted of 2 min immersion in 0.01M HCl, at room temperature, for 4 times; and toothbrushing with either of the abrasive suspensions (low and high, as previously determined by the radioactive dentin abrasivity method). Enamel and dentin surfaces were scanned at baseline, after treatment, at 5 days and at 10 days using an optical profilometer. Surface change (loss/gain) was determined by subtracting the treated area from the reference (protected) areas. Significance level of 5% was adopted for the statistical analysis.
Results: No differences were found among groups at baseline, regardless of substrate. After treatment, surface deposition was found for all test groups except for IC, which did not differ from C. For enamel, at day 5, FV, HS and SP had less surface loss than C and IC ($p<0.0008$), which did not differ from each other ($p=1.00$). At day 10, similar trend was observed except for FV, which showed surface loss similar to C, when brushed with high abrasive suspension. High abrasive caused more surface loss than low abrasive only for C at day 5 ($p=0.0117$) and 10 ($p=0.0162$). For dentin, at day 5, FV, HS and SP had less surface loss than C and IC ($p\leq0.0001$), which did not differ from each other ($p=1.00$). At day 10, HS and SP had less surface loss than C, IC, and FV ($p<0.0001$), and FV had less surface loss than C for low abrasive ($p=0.0009$). Overall, high abrasive had significantly more surface loss than low abrasive at 10 days ($p=0.0280$).Conclusion: HS was the most effective material protecting enamel and dentin from erosion-abrasion, followed by SP. FV offered limited protection, while no benefit was observed for resin infitrant IC.

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